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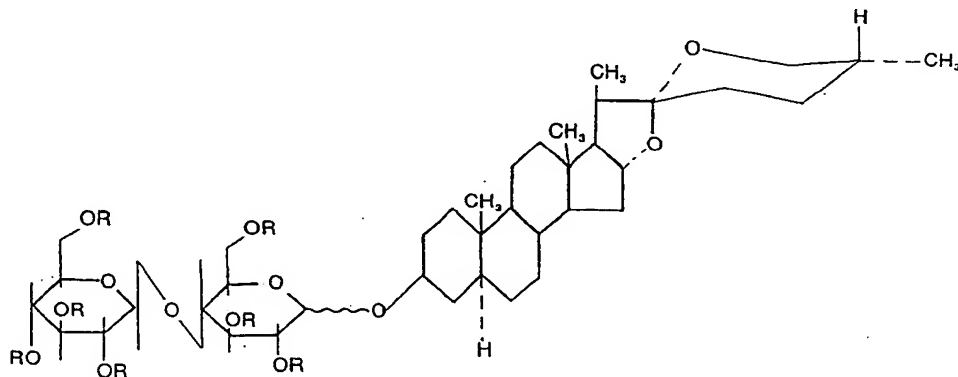
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㉖ **Tigogenin-cellobioside and its heptaacetate, methods for their preparation, and pharmaceutical compositions containing tigogenin-cellobioside.**

㉗ Tigogenin cellobioside is a potent inhibitor of cholesterol absorption and therefore useful in treating hypercholesterolemia and arteriosclerosis. Tigogenin cello-

bioside heptaacetate is useful as an intermediate in preparing tigogenin cellobioside. The two compounds are represented by the formula



wherein R is hydrogen or $-\text{C}(\text{O})\text{CH}_3$, either as a mixture of the α - and β -anomers or as the individual α - or β -anomer.

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TIGOGENIN-CELLOBIOSIDE AND ITS HEPTAACETATE,
METHODS FOR THEIR PREPARATION, AND PHARMACEUTICAL
COMPOSITIONS CONTAINING TIGOGENIN-CELLOBIOSIDE

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This invention relates to tigogenin-cellobioside, tigogenin-cellobioside heptaacetate and to the use of the former compounds for treatment of hypercholesterolemia and arteriosclerosis.

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Related Disclosures

French Patent 2,425,859 and U.S. Patent 4,260,603 describe certain sterol glycosides and spiroketal-steroid glucosides which have prostaglandin synthetase inhibiting activity.

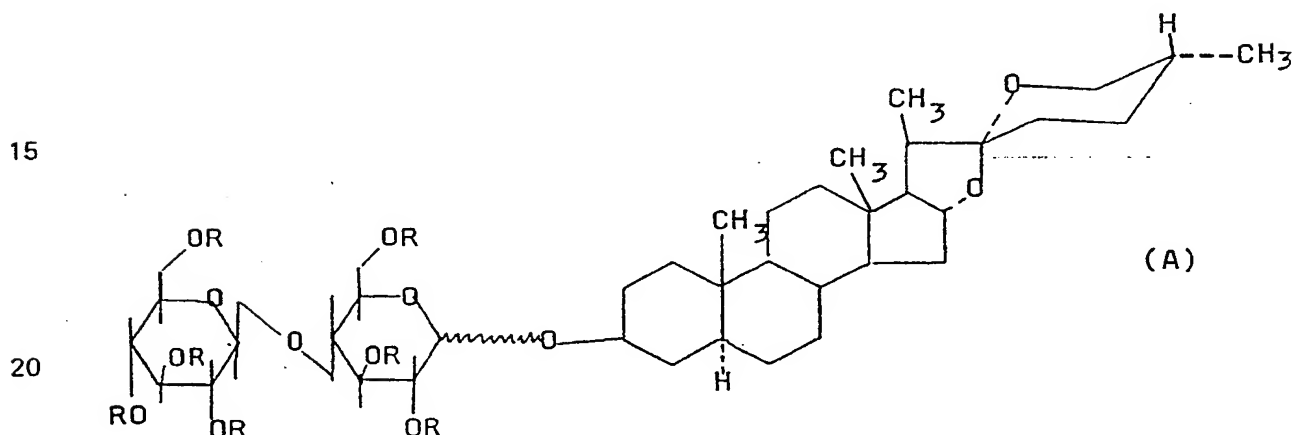
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Hemolytic properties of tigogenin
 α -L-rhampyranoside and tigogenyl β -maltoside are
 described in J. Pharm. Sci., 67(11):1589 (1978).

A neotigogenin diglucoside is taught as having
 5 hypocholesteremic activity in Khim.Farm.Zh., 15(9):55-60
 (1981).

SUMMARY

10 One aspect of this invention is a compound of formula



wherein R is hydrogen or $-C(=O)CH_3$ as an individual
 α - or β -anomer or a mixture thereof. The compound
 25 wherein each R is $-C(=O)CH_3$ is useful as an intermediate
 in the preparation of the compound wherein R is hydrogen.

Another aspect of this invention is the use of a
 compound of Formula (A) where R is hydrogen for treating
 hypercholesterolemia and arteriosclerosis.

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Still another aspect of this invention is a
 pharmaceutical composition comprising a therapeutically
 effective amount of a compound of Formula (A) where R is
 hydrogen in admixture with suitable pharmaceutically
 35 acceptable excipient.

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The last aspect of this invention is a process of making the compound of this invention, particularly the process for preparation of an α - or β -anomer.

DETAILED DESCRIPTION OF THE INVENTION

5 Definitions

As used hereinafter:

The wavy line in Formula (A) illustrates that the steroid is attached to the cellobioside moiety either above or below the plane of the six-membered ring to
10 which the steroid is attached.

" α -Anomer" means the compound wherein the steroid is attached below the plane of the six-membered ring.

" β -Anomer" means the compound wherein the steroid is attached above the plane of the six-membered ring.

15 "Treatment" covers any treatment of the disease in a mammal, particularly human, and includes:

(i) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it;

20 (ii) inhibiting the disease, i.e. arresting the development of said disease; or

(iii) relieving the disease, i.e. causing regression of the disease.

"Hypercholesterolemia", also known as
25 hypercholesteremia or hypercholesterinemia, means the presence of an abnormally large amount of cholesterol in the cells and plasma of the circulating blood.

"Arteriosclerosis" as used herein means a degenerative arterial sclerosis marked by hardening and
30 thickening of the vessel walls. The types of arteriosclerosis generally recognized are atherosclerosis, Monckerberg's arteriosclerosis, hypertensive arteriosclerosis, arteriolosclerosis or senile arteriosclerosis.

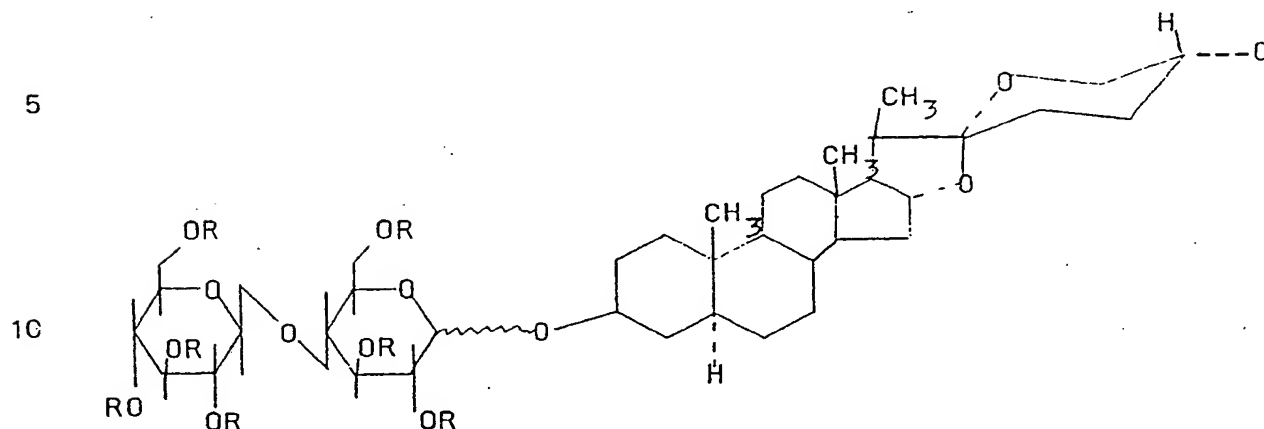
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Preferred Embodiments

This invention concerns a compound of the formula



wherein R is either hydrogen or $-\text{C}(\text{O})\text{CH}_3$,
 namely tigogenin cellobioside or tigogenin cellobioside
 15 heptaacetate, as individual α -anomers, β -anomers or a
 mixture thereof.

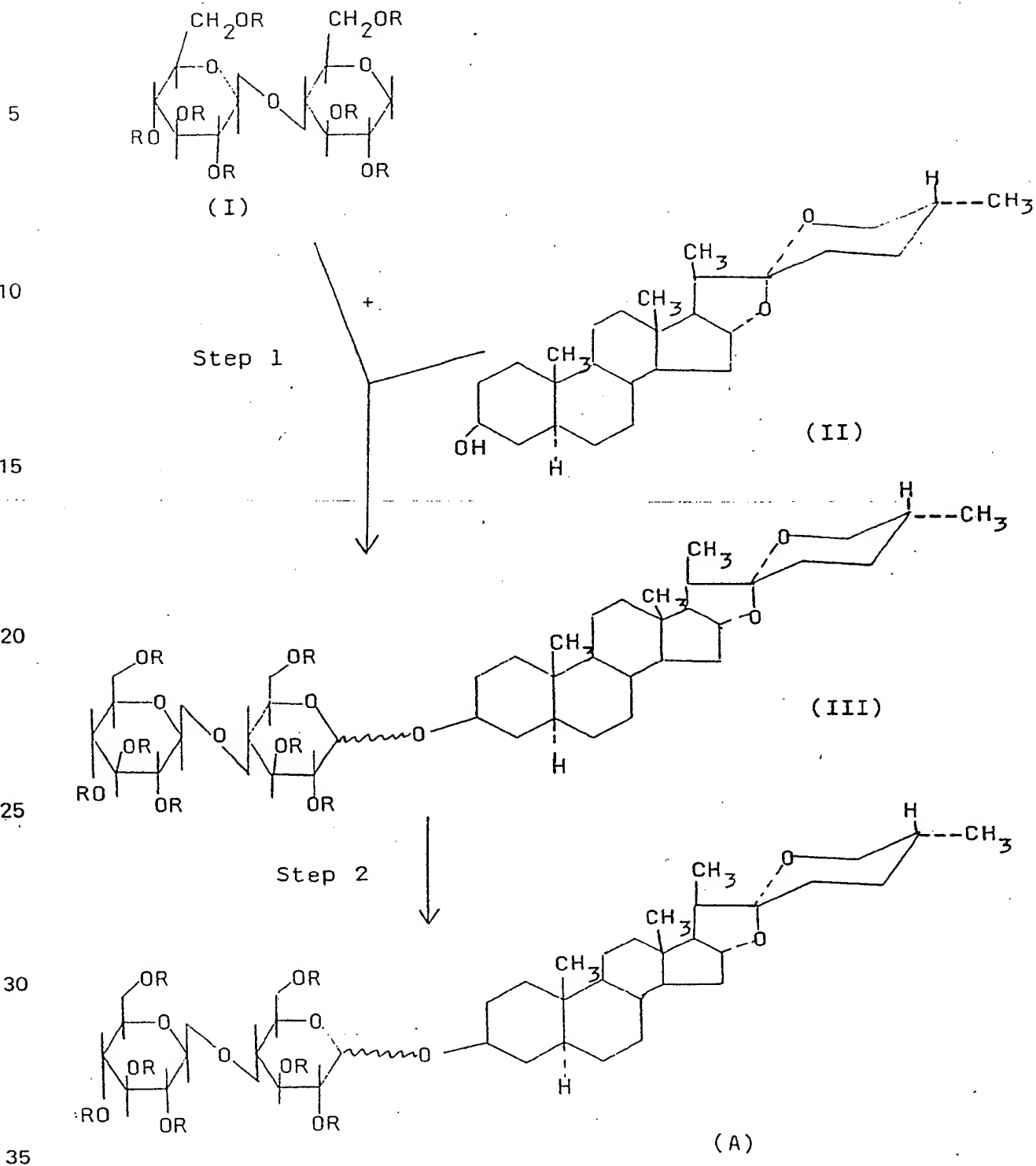
The preferred compounds are those wherein R is
 hydrogen, namely:

20 α -tigogenin cellobioside;
 β -tigogenin cellobioside; or
 the mixture of α - and β -tigogenin cellobioside.

Preparation Procedures

Reaction Scheme 1 illustrates the preparation of
 tigogenin cellobioside (A). In the formula (A) and in the
 25 following text the term tigogenin cellobioside is meant
 to include the mixture of α - and β -anomers of
 tigogenin cellobioside or individual α -anomer, or
 β -anomer.

In Reaction Scheme I, the X in the compound of
 30 Formula (I) is OR or Br, the R in formula (III) is
 $-\text{C}(\text{O})\text{CH}_3$ and the R in formula (A) is hydrogen.

REACTION SCHEME 1

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Reaction Scheme 1 illustrates processes of preparation of an individual α -anomer, an individual β -anomer or a nonseparated mixture of both. The starting compounds are cellobiose octaacetate (I where X is $-\text{OC}(\text{O})\text{CH}_3$) or bromocellobiose heptaacetate (I where X is Br) and tigogenin (II). All three processes where X is $-\text{OC}(\text{O})\text{CH}_3$ go through tigogenin cellobioside heptaacetate (III) as an intermediate and result in the same final compound (A).

By employing different starting materials and reaction conditions one obtains the individual α - and β -anomers (Procedures 1 and 2) or the mixture of both (Procedure 3).

Procedure 1

By using different solvents, one obtains the α - or the β -anomer. An article in Tetrahedron Letters, 28:1379 (1984) describes such solvent induced stereochemical modification. In this case, the use of acetonitrile gives the α -anomer. The use of the methylene chloride, on the other hand, gives β -anomer.

Step 1 Step 1 illustrates the preparation of cellobioside heptaacetate (III).

The starting materials, cellobiose octaacetate and tigogenin, are commercially available from Aldrich, Research Plus, or other sources.

Cellobiose octaacetate (I where X is $-\text{OC}(\text{O})\text{CH}_3$) is reacted with tigogenin (II) in an organic solvent, preferably acetonitrile for preparation of α -anomer and methylene chloride for preparation of β -anomer, in approximate amounts of 65:200:5000 wt:wt:vol. A metal chloride, preferably stannic chloride, is added over a period of 1-5 minutes, preferably 2 minutes. The reaction mixture is warmed to a temperature between 50-75°C, preferably to 65°C or to the temperature when the mixture becomes homogeneous.

That temperature is maintained for 10-60 minutes, preferably for 20 about minutes, then cooled to 20-40°C, preferably 30°C. The mixture is then submitted to
5 purification procedures by methods known in the art to give, depending on the solvent which is used, the α - or β -tigogenin cellobioside heptaacetate (III).

Step 2 Step 2 illustrates the preparation of
10 tigogenin cellobioside (A) by hydrolysis of compound (III).

Compound (III) is reacted with water and a mixture of organic solvents, preferably methylene chloride, triethylamine and methanol, in approximate amounts of 4:10:6:12:24 wt:v:v:v, at temperature of 20-80°C,
15 preferably at reflux, for about 2-10 hours, preferably for about 6 hours. Then, the reaction mixture is stirred overnight at temperature of 15-30°C, preferably at room temperature. The resulting material is evaporated, purified and separated by methods known in the art to
20 give α - or β -tigogenin-cellobioside (A), depending on which isomer of tigogenin-cellobioside heptaacetate was used in the Step 2.

Procedure 2

This is an alternative method for preparing the
25 individual α -and β -anomers.

Step 1

Bromocellobiose heptaacetate (I) where X is Br is prepared by reacting approximately equimolar quantities of cellobiose octaacetate with a brominating agent such
30 as titanium tetrabromide in a non-polar organic solvent such as a chlorinated hydrocarbon, particularly 1,2-dichloroethane, at a slightly elevated temperature which is generally below the boiling point of the solvent employed, e.g. 60-65°C. The reaction is complete in less
35 than about 10 hours, generally less than about 5 hours. By using purification procedures known in the art, one

obtains the compound of formula I where X is Br. This compound is then reacted with an approximately equimolar amount of tigogenin in the presence of a reaction promoter which is a heavy metal salt such as mercuric cyanide. This can be referred to as Koenigs-Knoor reaction. The resulting product is β -tigogenin cellobiose heptaacetate.

The α -anomer of tigogenin cellobiose heptaacetate is prepared by reacting a molar excess of bromocellobiose heptaacetate with tigogenin (about a 2:1 molar ratio) in the presence of diisopropylamine and tetraethylammonium bromide in a suitable halogenated hydrocarbon solvent such as methylene chloride. The reaction takes place at a temperature of about 10-40°C, preferable about 20-25°C, over a period of a few hours to several days, generally about 2 days or less. The α -anomer is then obtained using known purification techniques.

Step 2

The hydrolysis of the α or β -tigogenin cellobiose heptaacetate is carried out in accordance with the method set forth in Step 2 of Procedure 1.

Procedure 3

Procedure 3 illustrates the preparation of the mixture of predominantly β -tigogenin-cellobioside.

Step 1 Both tigogenin (I) and cellobiose octaacetate (II) are dissolved in an organic solvent, preferably in methylene chloride, in approximate amounts 4:80 w/v and 14:80 w/v, respectively. A metal salt, preferably stannic tetrachloride, is added to cellobiose octaacetate in an amount approximately equal to that of cellobiose octaacetate. The resulting solution is then added to the tigogenin solution and reacted for 1-8 hours, preferably for 3 hours, at a temperature of 15-30°C, preferably at room temperature. The mixture is washed with buffer, preferably with bicarbonate and the

gas which develops during the reaction is removed. The mixture is submitted to purification and separation by techniques known in the art such as TLC to yield the mixture of α - and β -tigogenin cellobioside
5 heptaacetate (III).

Step 2 An organic solvents/water mixture, preferably triethylamine/methanol/methylene chloride/water and the solution of heptaacetate (III), obtained above, is reacted under constant stirring for about 6-24
10 hours, preferably overnight at a temperature of 15-30°C, preferably at room temperature. The solvent is removed and the residue is submitted to purification and separation by techniques known in the art. The purified mixture is dialyzed in a tilting dialyzer (described in
15 Example 3) for 1-4 days, preferably for 3 days. The mixture is submitted to another purification procedure and extracted with an organic solvent, preferably heptane, in a soxhlet. The resulting residue is dried at 15 to 30°C, preferably at room temperature for 1-4 days,
20 preferably for 3 days, to yield a predominantly β -tigogenin-cellobioside.

The mixture of α - and β -tigogenin cellobioside is obtained by mixing the proportional amounts of individual α - and β - anomers.

25 Isolation and purification of the compounds and intermediates described herein can be effected, if desired, by any suitable separation or purification procedure such as, for example, filtration, extraction, crystallization, column chromatography, thin-layer
30 chromatography or thick-layer chromatography, or a combination of these procedures. Specific illustrations of suitable separation and isolation procedures can be had by reference to the examples hereinbelow. However, other equivalent separation or isolation procedures
35 could, of course, also be used.

UTILITY AND ADMINISTRATIONUtility

This invention relates to certain glycosides which are potent inhibitors of cholesterol absorption and are therefore primarily useful for treatment of hypercholesterolemia. Since the hypercholesterolemia is closely related to the development of generalized cardiovascular, cerebral vascular or peripheral vascular disorders, secondarily these compounds prevent the development of arteriosclerosis.

Cholesterol, which belongs to the body major plasma lipids, is highly soluble in fat but only slightly soluble in water. It is capable of forming esters with fatty acid and approximately 70% of the cholesterol present in plasma is in the form of cholesterol esters.

Cholesterol present in the body is either of endogenous or exogenous origin. Exogenous cholesterol is present in the diet and is absorbed slowly from the gastrointestinal tract into the intestinal lymph. Endogenous cholesterol, in a rather large quantity, is formed in the cells of the body. Essentially, all the endogenous cholesterol that circulates in the lipoproteins of the plasma is formed by the liver, but all other cells of the body can and do form at least some cholesterol.

The major plasma lipids, including cholesterol, do not circulate free in the plasma, but are bound to proteins and transported as macromolecular complexes called lipoproteins.

The plasma lipoproteins can be separated into four major classes:

- o chylomicrons;
- o very low density lipoproteins (VLDL);
- o low density lipoproteins (LDL); and
- o high density lipoprotein (HDL).

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Because of a varying ratio of lipid to protein, the densities of lipoproteins differ. The lipoproteins can be successfully separated by ultracentrifugation or by electrophoresis. The pathological hyperlipoproteinemias, which will be treated by the method of this invention, are classified on the basis of the pattern of lipoprotein abnormalities.

Depending on the plasma lipoprotein pattern, it is possible to classify patients with three types of hyperlipemia abnormalities: hypercholesterolemia, combined hyperlipemia and hypertriglyceridemia.

Hypercholesterolemia, combined hyperlipemia and hypertriglyceridemia occur commonly and involve two classes of lipoproteins (VLDL and LDL) for which there is a positive correlation between plasma concentration and the incidence of atherosclerosis.

For reference to the above, see Guyton, Medical Biology, 5th Ed., pp.926-927 (1976); The Merck Index, 13th Ed., pp.381-383 (1977) and Goodman and Gilman, The Pharmacological Basis of Therapeutics, 5th Ed., pp 744-747 (1975).

Inhibition of cholesterol absorption for compounds of this invention was studied in monkeys using the screening test described in J. Clin. Invest., 67:156 (1981). The inhibitory activity of a compound of this invention was compared to that of cholestyramine, a known antilipoproteinemic drug, and found to be five times more potent. The same results as those obtained with 2% cholestyramine were obtained with the 0.4% tigogenin-cellobioside. Since it is well known that the current treatment of hypercholesterolemia requires enormous daily dosages, this finding is of great importance. For example, the recommended adult dose of cholestyramine is 4 g three to four times daily which represents the total dose of 12-16 g of cholestyramine in admixture with

15-20 g of excipient. Thus, the total volume of the drug administered daily is between 27-36 g. On the other hand, the same effect has been obtained with the daily dose of only 0.8 g three to four times daily in a total
5 dose of 2.4-3.2 g in admixture with 3-4 g of excipient, i.e. the total volume of the drug which is expected to be administered daily is between 5.4-7.2 g per day.

The percentage of cholesterol absorption in rat was studied by using the test described in Am. J. Clin.
10 Nutr., 30:2061 (1977). When compared to other synthetic glycosides such as structurally related diosgenin-cellobioside, tigogenin-glucoside, diosgenin-glucoside or alfalfa saponins, compounds of the current invention had lower percentage of cholesterol
15 absorption and thus were more effective in removing the cholesterol from the plasma.

Administration

Administration of tigogenin-cellobioside can be via any of the accepted modes of administration suitable for
20 treatment of the hypercholesterolemia or arteriosclerosis. These methods include oral routes, parenteral routes such as intravenous, subcutaneous, intradermal, or intramuscular and other systemic routes of administration such as, for example, by suppositories.

25 The amount of tigogenin-cellobioside administered will, of course, be dependent on the subject being treated, on the severity of the affliction, on the manner of administration and on the judgment of the prescribing physician. However, an effective dosage is in the range
30 of 7 to 115 mg/kg/day, preferably 28 to 57 mg/kg/day. For an average 70 kg human, this would amount to 0.5 to 8 g/day, preferably 2-4 g/day, most preferably 2.4-3.2 g/day.

For oral administration, which is preferred, a
35 pharmaceutical composition takes the form of solutions,

suspensions, tablets, pills, capsules, powders, sustained release formulations and the like.

Parenteral route of administration is the administration of drugs to a patient by injection under
5 or through one or more layers of the skin or mucous membrane. Parenteral administration would preferably be reserved for crisis situations, wherein the subject is unable to swallow or administer the medication to himself.

Systemic administration via suppository is the
10 administration of the drug in a solid but readily meltable cone or cylinder made of a tigogenin-cellobioside and a suitable pharmaceutical excipient. Suppository must be suitable for insertion into a bodily passage or cavity, and is usually inserted
15 into the rectum. This way of administration would be preferred in the patient with severe ingestion disturbance such as repeated vomiting.

Pharmaceutical Composition

Depending on the intended mode of administration,
20 the pharmaceutical compositions may be in the form of solid, semi-solid or liquid dosage forms, such as, for example, tablets, pills, capsules, powders, liquids, suspensions, or the like, preferably in unit dosage forms suitable for single administration of precise dosages.

25 The pharmaceutical compositions will include a conventional pharmaceutical carrier or excipient and, tigogenin-cellobioside as an active ingredient. In addition, it may include other medicinal or pharmaceutical agents, carriers, adjuvants, etc.

30 A pharmaceutical composition may contain 0.1%-95% of tigogenin-cellobioside, preferably 1%-70%. In any event, the composition or formulation to be administered will contain a quantity of tigogenin-cellobioside in an amount effective to alleviate the signs of the subject being
35 treated, e. i. hypercholesterolemia or arteriosclerosis.

For solid pharmaceutical compositions, conventional non-toxic solid carriers include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose,
5 glucose, sucrose, magnesium carbonate, and the like.

Liquid pharmaceutically administerable compositions can be prepared by dissolving or dispersing, or otherwise preparing tigogenin-cellobioside, and mixing it optionally with a pharmaceutical adjuvant in a carrier,
10 such as, for example, water, saline, aqueous dextrose, glycerol, ethanol, and the like, to thereby form a solution or suspension.

For parenteral administration, such as, for example, intravenous injections, the tigogenin-cellobioside is
15 dissolved in a vehicle. Vehicle may be, for example, aqueous vehicle, such as sodium chloride injection, Ringer's injection, dextrose injection and others, water miscible vehicle, such as ethyl alcohol, polyethylene glycol of the liquid series or propylene glycol, or
20 nonaqueous vehicles such as a corn oil, peanut oil or sesame oil. Vehicle will be buffered to the proper pH in order to stabilize a solution against chemical degradation and formed in such a way as to control isotonicity of injection. Other substances may also be added as
25 antimicrobial or antioxidant agents.

A more recently devised approach for parenteral administration employs the implantation of a slow-release or sustained-release system, such that a constant level of dosage is maintained. See, e.g., U.S. Patent No.
30 3,710,795.

For systemic administration via suppository, the tigogenin-cellobioside may be formulated as suppositories using as the carrier traditional binders and carriers such as, for example, polyalkylene glycols, or
35 triglycerides. Such suppositories may be formed from

mixtures containing tigogenin-cellobioside in the range of 0.5%-10%; preferably 1-2%.

Methods of preparing various pharmaceutical compositions with a certain amount of active ingredient are known, or will be apparent, to those skilled in this art. For examples, see Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pennsylvania, 15th Edition (1975).

EXAMPLE 1

10 Preparation of α -Tigogenin Cellobioside

1. Preparation of α -Tigogenin Cellobioside Heptaacetate

A 12 l three neck round bottom flask fitted with a mechanical stirrer and a 500 ml dropping funnel was flushed with nitrogen and charged with 650 g (0.96 moles) of cellobiose octaacetate, 200 g (0.48 moles) of
15 tigogenin, and 5.1 of acetonitrile. A total of 250 g (0.96 moles) of stannic chloride was then added over two minutes through the dropping funnel. The reaction mixture was warmed on a steam bath, until it became
20 homogeneous (65°C). The 65°C temperature was maintained for 20 min, then the mixture was cooled to 30°C. 4 litres of saturated aqueous sodium bicarbonate solution was added carefully and the mixture was vigorously stirred for 90 minutes. The layers were separated and
25 the aqueous phase reextracted with 4 litres of methylene chloride. The combined organic phases were dried over magnesium sulphate and evaporated under reduced pressure to a gum. The gum was passed through a silica gel column (1.5kg) eluted with 25% methylene chloride/hexane (v/v).
30 The fraction which was obtained was evaporated under vacuum. The residue was crystallized from ethanol to give 120 g (24% of theory yield) of α -tigogenin cellobioside heptaacetate.

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2. Preparation of α -Tigogenin-Cellobioside

A mixture of 38.2 g of α -tigogenin cellobioside heptaacetate in 60 ml of methylene chloride, 240 ml of methanol, 120 ml of triethylamine and 100 ml of water was
5 refluxed for 6 hours and then stirred overnight at room temperature. The reaction mixture was evaporated under reduced pressure to a thick paste which was washed with water and hexane and dried. The resulting material was chromatographed on a column of 3 kg of silica gel using
10 10% methanol/methylene chloride (v/v) to elute desired material. After evaporation of the fraction which contained α -tigogenin cellobioside, the residue was stirred in boiling isopropanol, cooled, and collected to give 13.9 g of α -tigogenin cellobioside.
15 NMR data for α - and β -tigogenin-cellobioside are given in Table I which follows Example 3.

EXAMPLE 2

Preparation of β -Tigogenin-Cellobioside

20 1. Preparation of β -Tigogenin-Cellobioside Heptaacetate
A 12 liters three neck round bottom flask fitted with a mechanical stirrer and a 40 ml dropping funnel is flushed with nitrogen and charged with 650 g (0.96 moles) of cellobiose octaacetate, 200 g (0.48 moles) of
25 tigogenin, and 500 ml of methylene chloride. A total of 250 g (0.96 moles) of stannic chloride is then added over two minutes through the dropping funnel. The reaction is warmed on a steam bath, and refluxed for 20 min, then cooled to 30°C. 4 litres of saturated aqueous sodium
30 bicarbonate solution is added carefully and the mixture is vigorously stirred for 90 minutes. The layers are separated and the aqueous phase reextracted with 4 litres of methylene chloride. The combined organic phases are dried over magnesium sulphate and evaporated under
35 reduced pressure to a gum. The gum is passed through a

silica gel column (1.5 kg) eluting with 25% methylene chloride/hexane (v/v). The fraction containing β -tigogenin cellobioside is evaporated under vacuum. The residue is crystallized from ethanol to give

5 β -tigogenin cellobioside heptaacetate.

2. Preparation of β -Tigogenin-Cellobioside

A mixture of 38.2 of β -tigogenin cellobioside heptaacetate in 60 ml of methylene chloride, 240 ml of methanol, 120 ml of triethylamine and 100 ml of water, is
10 refluxed for 6 hours, then stirred overnight at room temperature. The reaction mixture is evaporated under reduced pressure to a thick paste which is washed with water and hexane and dried. The resulting material is chromatographed on a silica gel column of 3 kg of silica
15 gel using 10% methanol/methylene chloride (v/v) to elute desired material. After evaporation of the appropriate fractions, the residue is stirred in boiling isopropanol, cooled, and evaporated to give β -tigogenin cellobioside.

EXAMPLE 3

Preparation of β -Tigogenin-Cellobioside

General Information and Procedures

Tigogenin was obtained from Research Plus.

Cellobiose octaacetate was obtained from Aldrich.

Tilting dialyzer A tilting dialyzer was built
25 using a large container provided with a siphon in such a way that the water level was continuously rising and falling.. The dialysis bags were tied to a vertical metal rod midway between the upper and lower water levels. The tilting bags moved the powdered material and
30 increased its contact with water.

Experimental Procedure

Tigogenin 41.9 g of tigogenin (100 mmoles) was dissolved in 800 ml of water-free methylene chloride.

Water-free methylene chloride Water-free methylene
35 chloride was prepared by adding the $MgCl_2$ to a

commercially obtained methylene chloride. The solution was stirred for 30 min and filtered.

Cellobiose octaacetate 135 g of cellobiose octaacetate (200 mmoles) was dissolved in 800 ml of water-free methylene chloride and 25 ml of stannic tetrachloride (200 mmoles) was added. The mixture was stirred for 10 minutes.

The cellobiose octaacetate mixture, obtained above, was introduced into a separatory funnel and added dropwise to a solution of tigogenin, obtained above, at an approximate rate of 150 ml/min. The mixture was stirred for 3 hours. The resulting solution was poured into 4 liter separatory funnel containing 500 ml of saturated bicarbonate solution saturated with methylene chloride. 500 ml of water saturated with methylene chloride was added and the gas was allowed to escape. The solution was mixed by inversion and separated into an organic and water phases. Lower phases were transferred to a separatory funnel, washed twice with water saturated with methylene chloride and, after separation, the upper phase was aspirated and discarded. The remaining phase was washed twice with 250 ml of water, separated, and again the upper phase was discarded. The lower phase was washed twice with water, separated and again the lower phases were collected and evaporated to 100-200 ml at approximately 50°C under vigorous stirring and nitrogen atmosphere to give predominantly β -tigogenin cellobioside heptaacetate.

1400 ml of a mixture containing triethylenamine/methanol/water (1:2:1) was added slowly and under constant stirring to the solution of the above tigogenin cellobioside heptaacetate. The mixture was stirred overnight. Methylene chloride was removed at 30°C under vacuum. Three liters of water was added and the mixture was left in the refrigerator for 1 to 2 hours.

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Afterwards, the mixture was centrifuged at 4000 RPM for 20 min, precipitate dried at room temperature, crushed, dissolved in small amount of methanol and water, and dialyzed for 3 days in tilting dialyzer against running tap water. The resulting mixture was filtered, dried at room temperature, crushed into fine powder and extracted in Soxhlet (jacketed at 40°) with heptane for 3 days. β -tigogenin cellobioside which remained in the thimble was removed and dried at room temperature for 2 to 3 days to evaporate heptane. This procedure yielded 30 to 40% of β -tigogenin cellobioside.

The ^1H NMR spectra of $1'\alpha$ - and $1'\beta$ -tigogenin cellobioside were measured on the Bruker WM300 Fourier transform NMR spectrometer in d_6 DMSO solution with reference to internal tetramethylsilane.

Table-I

300 MHz ^1H NMR Data in d_6 DMSO

	<u>Assignments</u>	<u>Chemical Shifts in ppm</u>	<u>No. of Protons</u>	
		<u>Cellobioside</u>		
		<u>α-tigogenin</u>	<u>β-tigogenin</u>	
20	16 α	4.28m	4.25m	1
	18	0.72s	0.72s	3
	19	0.78s	0.78s	3
	21	0.89 d,	0.89	3
25		d, J=6.8Hz	d, J=6.8Hz	
	26	0.74	0.73	3
		d, J=6.3Hz	d, J=6.3Hz	
	1'	4.78	4.29	1
		d, J=3.5Hz	d, J=7.9Hz	
30	1''	4.23	4.23	1
		d, J=7.6Hz	d, J=7.6Hz	

J = coupling constant

s = singlet

d = doublet

m = multiplet

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EXAMPLE 4Effect of Tigogenin Cellobioside on Plasma Cholesterol

This example illustrates the effect of tigogenin cellobioside on plasma cholesterol in monkeys *Macaca fascicularis*. The procedure is described in J. Clin. Invest., 67:156 (1981).

Experimental procedureExperimental Regimen

The animals were divided into groups I and II.

10 Group I was treated with 2% cholestyramine, Group II was treated with 0.4% tigogenin cellobioside. Each group was divided into two subgroups. Animals in the first subgroup served as cholesterol controls, i.e. they received no drug treatment but were fed with a
15 cholesterol diet. Animals in the second subgroup received a drug treatment and were also fed with a cholesterol diet.

Subgroup 1: Before the beginning of the experimental regimen, the control blood was obtained from
20 all animals in both groups and a total cholesterol and a high density lipoprotein cholesterol were determined. Then the animals were fed the cholesterol diet. After three weeks on the diet without treatment, the blood from treated monkeys was collected and a total cholesterol and
25 a high density lipoprotein cholesterol were determined.

Subgroup 2: Before the beginning of the regimen of subgroup 2, the animals from subgroup 1 were fed a cholesterol-free, semipurified diet for five weeks. After five weeks on the cholesterol-free diet, the
30 control blood was again obtained from all animals and a total cholesterol and a high density lipoprotein cholesterol were determined. Then, the animals were put on a cholesterol diet combined with drug treatment. After three weeks of the cholesterol diet and of the
35 appropriate treatment, the blood from all monkeys was

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collected and a total cholesterol and a high density lipoprotein cholesterol were determined.

Animals and diet

Twelve adult female cynomolgus (Macaca fascicularis) were fed for 3 weeks the cholesterol-free semipurified diet (SPD) of the following content:

<u>Ingredient</u>		<u>g/100 g of diet</u>
	casein	18
	sugar	30
10	honey	10
	Alphacel	12
	butter	3
	coconut oil	8.5
	safflower oil	2.5
15	vitamins (OWP)	2
	salts (Hegsted IV)	4
	vitamin D-3 (2,000 IU/ml	0.2 ml
	banana (wet weight)	10

20	proteins (% calories)	20.6
	fat (% calories)	33.5
	carbohydrate (% calories)	45.9

This diet can optionally contain 0.118 g/100g of diet or 0.35 mg/kCal of cholesterol.

The experimental protocol is outlined and the results are summarized in Table A.

Results (summarized in Table A)

The monkeys were assigned to two groups (I and II, each of six monkeys) according to their serum cholesterol response; they were stratified and assigned randomly, thus resulting in groups with similar elevations of cholesterolemia.

Control 1, Sample 1.

Control venous blood was obtained for analysis and the mean values of the serum cholesterol were determined to be 240 mg/dl for Group I and 228 mg/ml for Group II.

Then, the monkeys were offered semipurified diet containing 0.1% cholesterol for 3 weeks. At the end of this period, blood sample 2 was obtained.

Cholesterol Diet, Sample 2

After three weeks on the cholesterol diet, the mean value of cholesterol, was 324 mg/dl in Group I and 316 mg/dl in Group II. The level of cholesterol was significantly higher in samples 2, with degree of significance = 0.01.

The monkeys were again given semipurified diet without cholesterol for 5 weeks and bled at the end of this period.

Control 2, Sample 3

After five weeks on the cholesterol-free diet, the level of cholesterol in both groups decreased to 221 mg/dl in Group I and to 189 mg/dl in Group II.

The animals were then given semipurified diet with 0.1% cholesterol and either 2% cholestyramine (Group I) or 0.4% cellobiose-tigogenin (Group II) for 3 weeks. At the end of this period, sample 4 was obtained.

Cholesterol Diet and Drug Treatment, Sample 4

After three weeks on the cholesterol diet combined with the drug treatment, the values of cholesterol in Group I was 226 mg/dl and 202 mg/dl in Group II.

Results shown in Table A demonstrate that both drugs prevented the expected rise in serum cholesterol. There were small, nonsignificant elevations in high-density lipoprotein-cholesterol associated with drug intake. See Samples 1, 2, 3 and 4 in HDL cholesterol section of the Table A. Although the changes in the triglyceride levels would be a natural response to the ingestion of a

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high-cholesterol diet, no changes were found. This indicates that both drugs prevented the increase in low-density lipoprotein cholesterol.

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Table A Serum cholesterol (mg/dl; mean \pm SD)^a

Period	Sample	Control 1	diet	<u>Total Cholesterol</u>		<u>HDL cholesterol</u>	
				Control 2	+ drug	Control 1 diet	Control 2 + drug
GROUP I N=6	Drug: 2% cholestyramine	1	2	3	4	1	2 3 4
		240 \pm 19	324 \pm 24	221 \pm 19	226 \pm 16	126 \pm 12	148 \pm 17 155 \pm 9 166 \pm 8
		paired t test					
		P: vs column 2	0.01	--	0.01	NS	-- NS
GROUP II N=6	Drug: 0.4% cellobiose-tigogenin	vs column 1	--	0.01	NS	NS	0.02
		vs column 3			NS		0.05
							NS
GROUP II N=6	Drug: 0.4% cellobiose-tigogenin	228 \pm 19	316 \pm 21	189 \pm 10	202 \pm 8	104 \pm 12	139 \pm 14 148 \pm 10 163 \pm 7
		paired t test					
		p: vs column 2	0.01	--	0.01	0.05	-- NS
		vs column 1	--	0.01	0.05	--	0.05 0.05 0.01
		vs column 3				NS	NS

^aAbbreviations: HDL-cholesterol, high-density lipoprotein cholesterol; N, number of animals; NS, not significant.

EXAMPLE 5Effect of Synthetic Glycosides on Intestinal
Absorption of Cholesterol in Rats

5 This example compares the intestinal absorption of cholesterol in rats treated with tigogenin cellobioside or other synthetic glycosides. The method used for this experiment is described in detail by Malinow et al., Am. J. Clin. Nutr., 30:2061 (1977).

10 Experimental Protocol

2-mg bolus of 4[¹⁴C]cholesterol was given intragastrically to rats. Feces were collected for 72 hours, and the fecal excretion of labeled neutral steroids was determined.

15 Rats were divided into three groups I-III.

In Groups I and II the animals were divided into subgroups of 6 rats. The first subgroup served as control and never received any treatment. The second and third subgroups were treated with either a compound of this invention or with one of the other, closely related compounds. Thus, in Groups I and II, the rats were treated with tigogenin cellobioside, diosgenin cellobioside, tigogenin glucoside and diosgenin glucoside.

25 Results summarized in Table B confirm that the tigogenin cellobioside decreased the intestinal absorption of cholesterol more markedly than structurally similar compounds diosgenin cellobioside, tigogenin glucoside and diosgenin glucoside. Tigogenin cellobioside was significantly more effective than closest related diosgenin cellobioside (see Group I, subgroups 2 and 3). When compared to control group, tigogenin cellobioside decreased the cholesterol absorption by almost 50% (with significance of = 0.001).
30 The tigogenin cellobioside was also more effective than
35

tigogenin glucoside and diosgenin glucoside (see Group II, subgroups 2 and 3, and compare Group I and II).

Group III has two subgroups with 12 animals in each. An inhibition of the cholesterol absorption in rats treated with tigogenin cellobioside was compared to the inhibition of the cholesterol absorption in nontreated controls.

Similarly to Group I, wherein treatment with tigogenin cellobioside inhibited absorption of cholesterol by 47% (No=6), in Group (III) the treatment with tigogenin cellobioside inhibited absorption of cholesterol by 41.5% (No=12). And, the absorption of cholesterol in experimental rats in Group (I) was only 53% and the absorption of cholesterol in in Group (III) was only 58.5% of the absorption of cholesterol in control group.

Table B

Effects of Synthetic Saponins on
Cholesterol Absorption in Rats

					P		
	<u>Series</u>	<u>Treatment</u>	<u>Number of Animals</u>	<u>Dose (mg)</u>	<u>Cholesterol absorption (% I.D.)</u>	<u>t test vs control</u>	<u>Relative Absorption</u>
25	I	none	6	0	74.8+1.6		100
		C-T	6	14	39.6+1.8	0.001	53
		C-D	6	14	53.7+1.3	0.001	72
	II	none	6	0	74.6+2.3		100
		G-T	6	14	46.2+1.8	0.001	62
30		G-D	6	14	52.6+3.7	0.001	71
	III	none	12	0	75.2+1.8		100
		C-T	12	14	43.6+1.6	0.001	58.5

Means \pm SD. Abbreviations: C-D, cellobiose diosgenin; C-T, cellobiose tigogenin; G-D, glucose diosgenin; G-T, glucose tigogenin.

EXAMPLE 6Preparation of the β -anomer of tigogenin cellobioside

5 5.5g (15 meq) of titanium tetrabromide was transferred under anhydrous conditions to a mixture of 10.2g (15meq) of cellobioseoctaacetate in 150 ml 1,2-ethylene dichloride (dried over sieves). The mixture was stirred over a 60-65°C oil bath for 5 hr. The mixture was cooled using an ice bath to room temperature then washed
10 consecutively with 50 ml ice water, then 50 ml cold sat. NaHCO_3 , then 50 ml water. The resulting, washed mixture was then dried using MgSO_4 , filtered and stripped using a warm water bath, finally using a rotary evaporator with a pump. Eleven grams of a solid residue
15 was recovered.

To this residue was added 2.9g (7.3meq) of tigogenin, 100ml of acetonitrile, and 7.6g (30meq) of mercuric cyanide, and the resulting mixture was heated under N_2 using a 60-65°C oil bath with vigorous
20 stirring for 1 1/2 hours (the mixture became homogeneous after 30 min.), then overnight. Solvent was removed from the reaction mixture using a rotary evaporator, the resulting material was dissolved in dichloromethane, and the insolubles were filtered off. The organic solution
25 was washed sequentially with sat. KHCO_3 30% KI solution and water. The aqueous extracts were combined and back extracted with dichloromethane. The organic phases were combined, dried over MgSO_4 and stripped. 4.9g of crystals of β -tigogenin cellobioside heptaacetate were
30 obtained by recrystallizing from ethanol. By recrystallizing from ethanol, 3.9g of crystals of the β -anomer were obtained.

The β -tigogenin cellobiose heptaacetate so obtained is hydrolyzed in accordance with the procedure set forth
35 in Example 2, part 2 to give β -tigogenin cellobiose exhibiting an NMR spectrum essentially the same as that shown in Table I of Example 3.

EXAMPLE 7Preparation of the α -anomer of tigogenin cellobioside

This reaction is a modification of the procedure presented by R.U. Lemieux, et al, J. Am. Chem. Soc., 97 4056 (1975).

A mixture of 10.7g (15mmole) of bromocellobiose heptaacetate (prepared as in Example 6), 1.95g (15mmole) of diisopropylamine, 3.15g (15mmole) of tetraethylammonium bromide, and 3.0g (7.5mmole) of tigogenin in 100ml of methylene chloride is stirred at room temperature for two days. The reaction mixture is then washed sequentially with water and 1N HCl and dried over sodium sulfate. After filtration and evaporation of the solvent at reduced pressure, the residue is purified by crystallization from ethanol to give the α -anomer of tigogenin cellobioside heptaacetate which in turn is hydrolyzed in accordance with the method of Example 2 to give the α -anomer of tigogenin cellobioside having NMR data essentially the same as presented in Table I of Example 3.

EXAMPLE 8Separation of the α - and β -anomers of tigogenin cellobioside heptaacetate.

A mixture of the α - and β -anomers is prepared by mixing equimolar amounts of the individual isomers or by reacting a mixture of the α - and β -anomers of tigogenin cellobioside with a appropriate acetylating material. The individual α - and β -anomers of tiogenin cellobioside heptaacetate are separated by micro TLC methods using a 60:40 mixture of ethylacetate and hexane on silica gel. Once the anomers are separated, an individual anomer is extracted from the TLC plate.

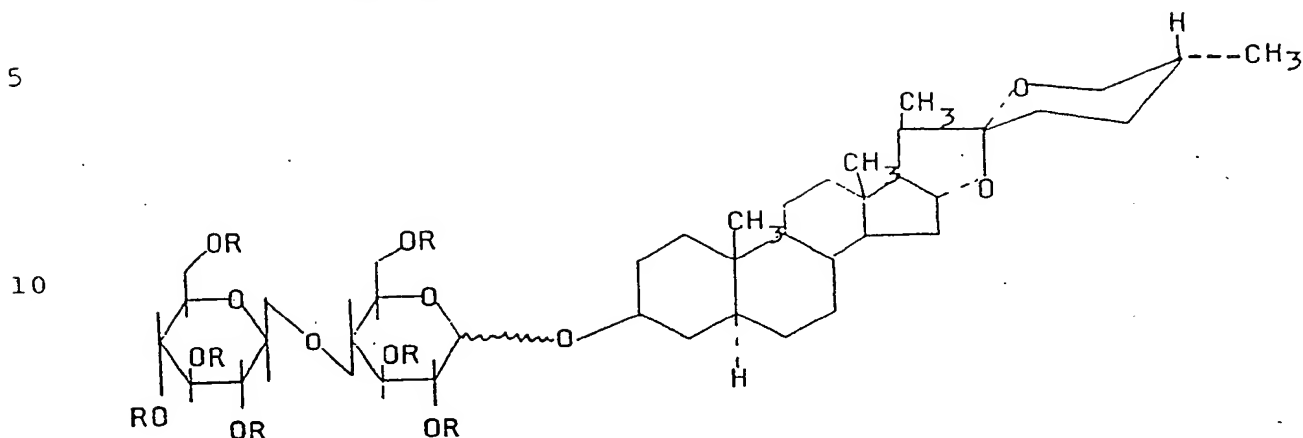
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CLAIMS

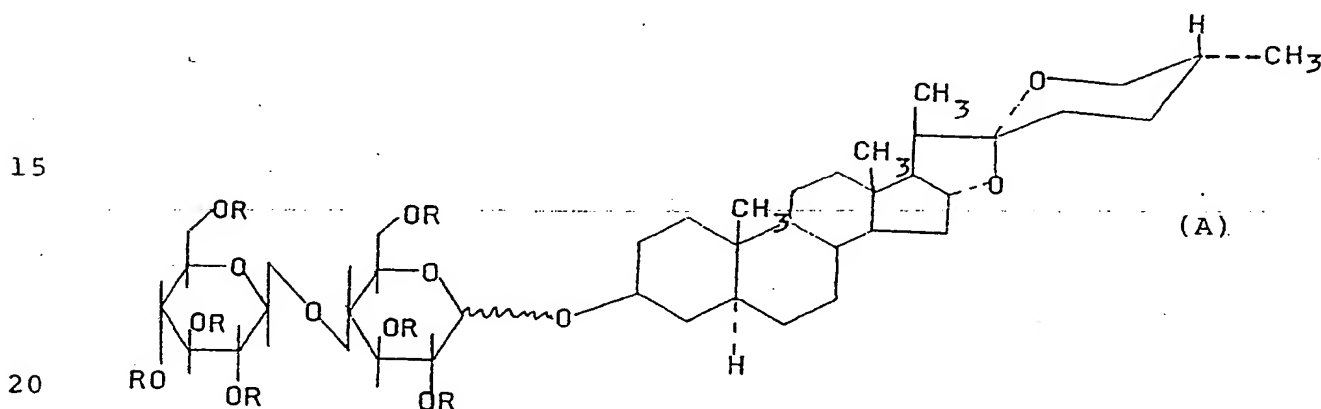
1. A compound of the formula



15 wherein R is hydrogen or $-C(O)CH_3$.

2. The compound of claim 1 wherein R is hydrogen and the compound is α -anomer, namely α -tigogenin cellobioside.
- 20 3. The compound of claim 1 wherein R is hydrogen and the compound is the β -anomer, namely β -tigogenin cellobioside.
4. The compound of claim 1 wherein R is $-C(O)CH_3$ and the compound is an α -anomer, namely α -tigogenin cellobioside
- 25 heptaacetate.
5. The compound of claim 1 wherein R is $-C(O)CH_3$ and the compound is a β -anomer, namely β -tigogenin cellobioside heptaacetate.
- 30 6. A pharmaceutical composition useful for treatment of hypercholesterolemia and arteriosclerosis in mammals which composition comprises a pharmaceutically effective amount of a compound of claim 1 in admixture with a pharmaceutically
- 35 acceptable excipient.

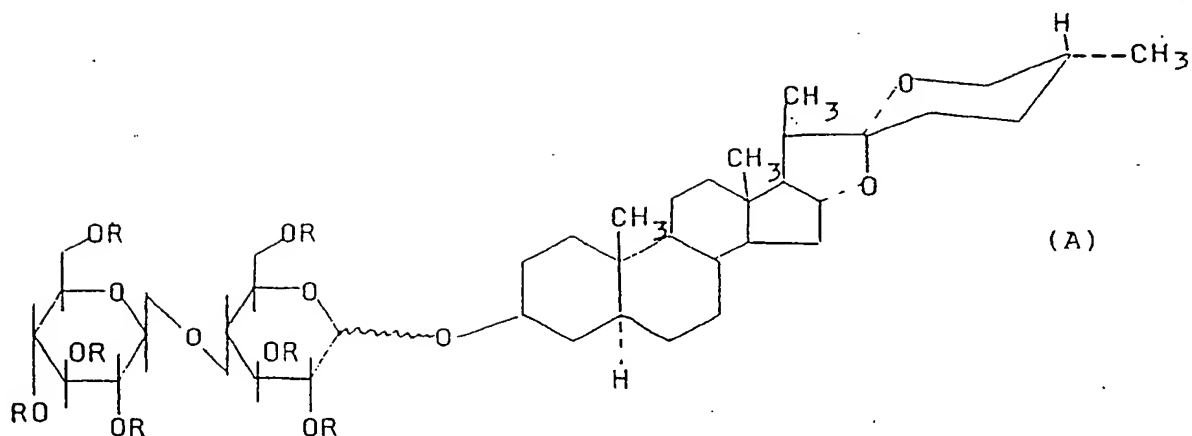
7. The composition of claim 6 wherein R of Formula (A) is hydrogen and the compound is the α -anomer, namely α -tigogenin cellobioside.
8. The composition of claim 6 wherein R of Formula (A) is hydrogen and the compound is the β -anomer, namely β -tigogenin cellobioside.
9. A process for the preparation of a compound of Formula (A)



wherein R is hydrogen, as a mixture of its α - and β -anomers or as the individual α -anomer or β -anomer, which process comprises

- hydrolysing a compound of Formula (A) wherein R is $-\text{C}(\text{O})\text{CH}_3$ to form a compound of Formula (A) wherein R is hydrogen and optionally
- extracting the α -anomer or β -anomer of a compound of Formula (A) wherein R is hydrogen.

10. A process according to claim 9 wherein said hydrolysis is effected with water in an organic solvent.
11. A process for the preparation of Formula (A)

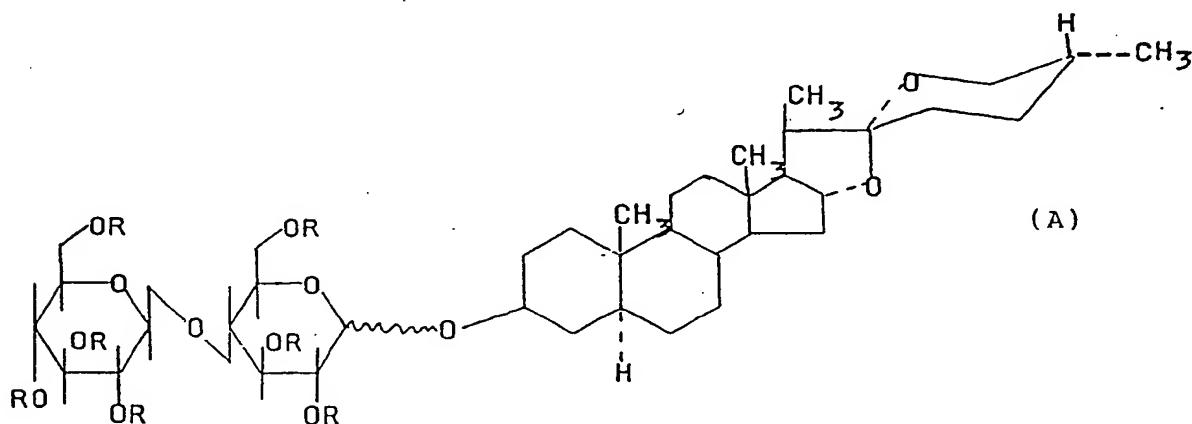


wherein R is $-\text{C}(\text{O})\text{CH}_3$, as a mixture of its α - and β -anomers or as the individual α -anomer or β -anomer, which process comprises

reacting cellobiose octaacetate or bromocellobiose heptaacetate with tigogenin in an organic solvent in the presence of a metal salt and optionally

extracting the α -anomer or β -anomer of a compound of Formula (A) wherein R is $-\text{C}(\text{O})\text{CH}_3$.

12. A process for the preparation of an anomer of a compound of Formula (A)



wherein R is $-\text{C}(\text{O})\text{CH}_3$, which process comprises extracting the α -anomer or the β -anomer from a mixture of the two anomers.

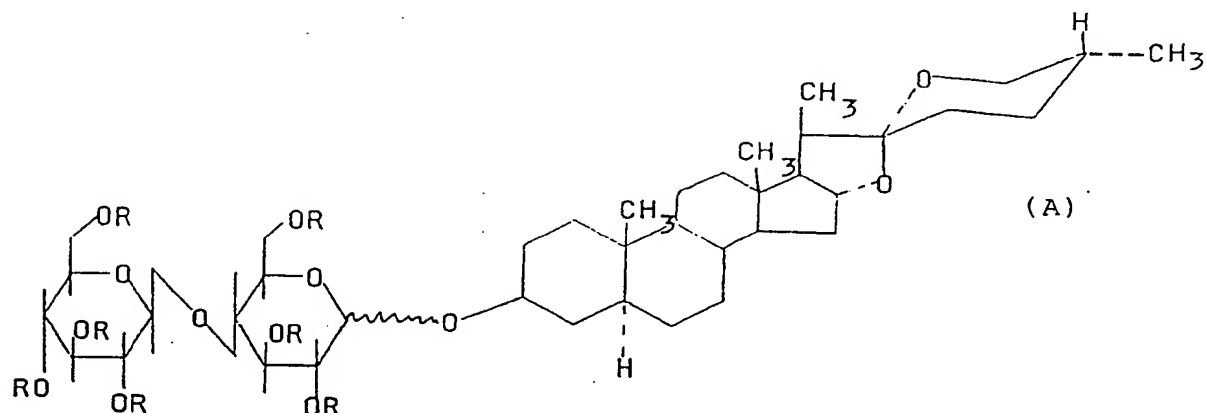
CLAIMS

1. A process for the preparation of a compound of Formula (A)

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wherein R is hydrogen, as a mixture of its α - and β -anomers or as the individual α -anomer or β -anomer, which process comprises

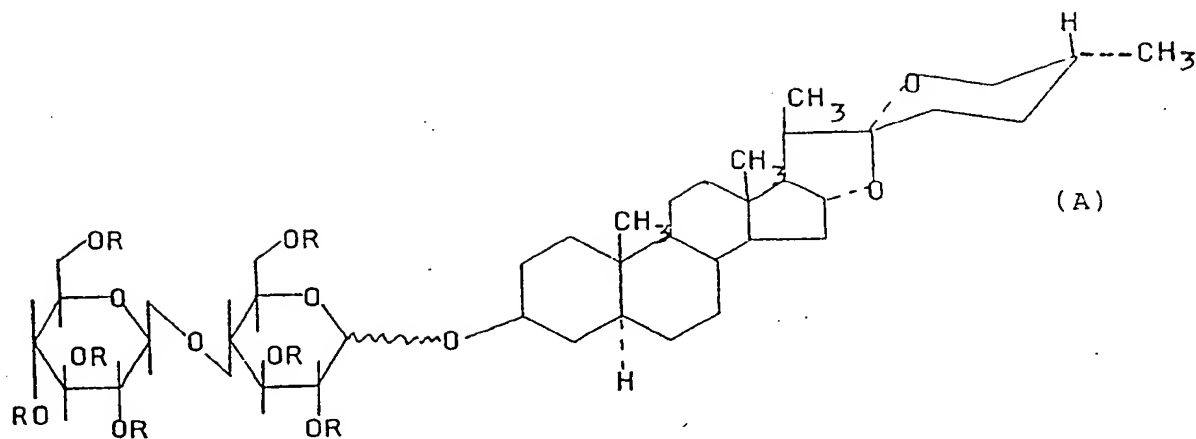
hydrolysing a compound of formula (A) wherein R is
20 $-C(O)CH_3$ to form a compound of Formula (A) wherein R is hydrogen and optionally

extracting the α -anomer or β -anomer of a compound of Formula (A) wherein R is hydrogen.

- 25 2. A process according to claim 1 wherein the hydrolysis is effected with water in an organic solvent.

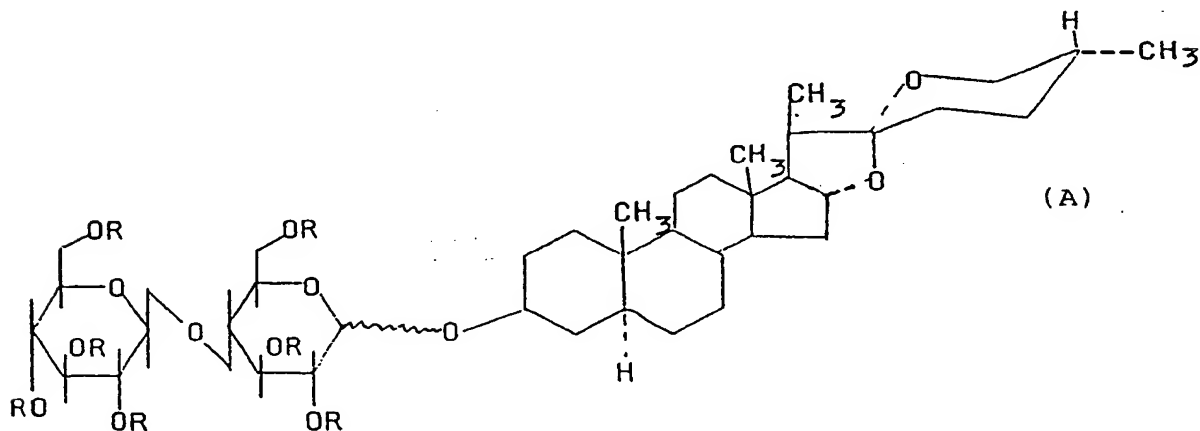
3. A process for the preparation of an anomer of a compound of Formula (A)

30



wherein R is hydrogen or $-C(O)CH_3$, which process comprises extracting the α -anomer or the β -anomer from a mixture of the two anomers.

4. A process for the preparation of a compound of Formula (A)



wherein R is hydrogen or $-C(O)CH_3$, as a mixture of its α - and β -anomers or as the individual α -anomer or β -anomer, which process comprises

(a) reacting cellobiose octaacetate or bromocellobiose heptaacetate with tigogenin in an organic solvent in the presence of a metal salt, and

(b) optionally extracting the α -anomer or β -anomer of

a compound of Formula (A) wherein R is $-C(O)CH_3$,

(c) optionally hydrolysing a product of step (a) or step (b) to form a compound of Formula (A) in which R is hydrogen,

5 (d) optionally extracting the α -anomer or β -anomer of a compound of Formula (A) from the product of step (c).

5. A process of any one of claims 1 to 4 wherein the product compound is α -tigogenin cellobioside.

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6. A process of any one of claims 1 to 4 wherein the product compound is β -tigogenin cellobioside.

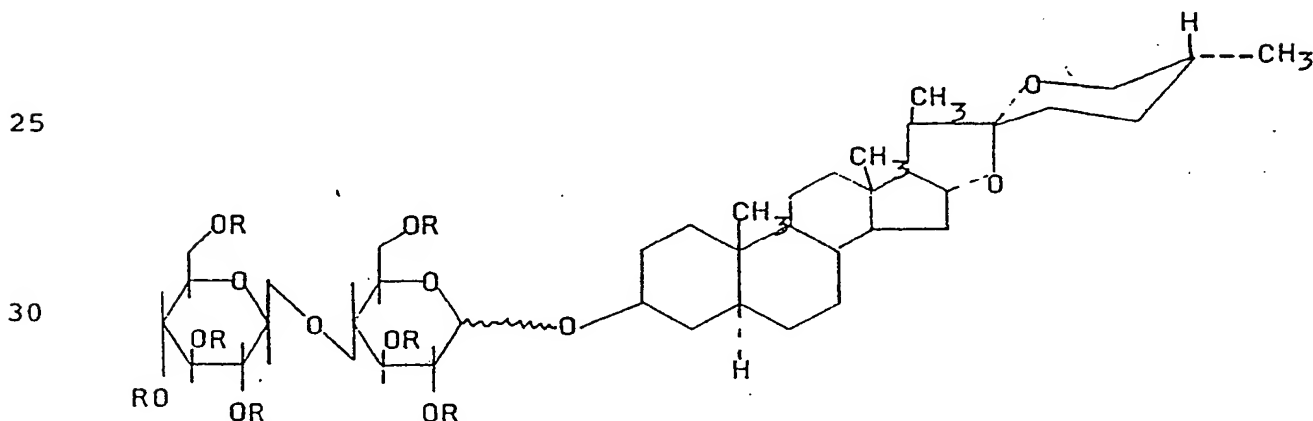
7. A process of claim 3 or claim 4 wherein the product compound is α -tigogenin cellobioside heptaacetate.

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8. A process of claim 3 or claim 4 wherein the product compound is β -tigogenin cellobioside heptaacetate.

9. A process which comprises mixing a compound of the formula

20



wherein R is hydrogen, as a mixture of its α - and β -anomers or as the individual α - or β -anomer, with a pharmaceutically acceptable excipient to produce a pharmaceutical composition.

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12 EUROPEAN PATENT APPLICATION

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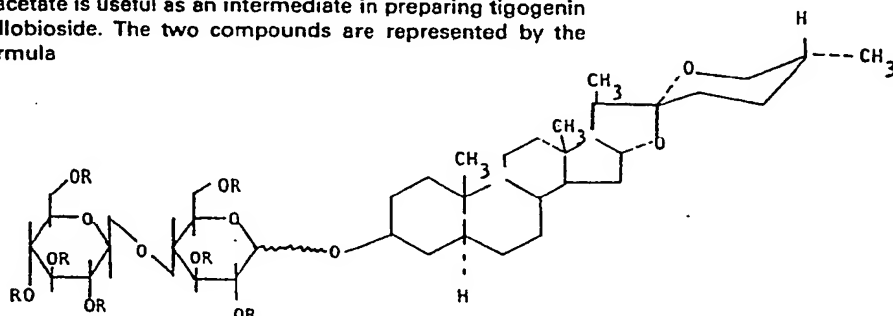
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64 Tigogenin-cellobioside and its heptaacetate, methods for their preparation, and pharmaceutical compositions containing tigogenin-cellobioside.

67 Tigogenin cellobioside is a potent inhibitor of cholesterol absorption and therefore useful in treating hypercholesterolemia and arteriosclerosis. Tigogenin cellobioside heptaacetate is useful as an intermediate in preparing tigogenin cellobioside. The two compounds are represented by the formula



wherein R is hydrogen or $-C(O)CH_3$, either as a mixture of the α - and β -anomers or as the individual α - or β -anomer.



European Patent
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EUROPEAN SEARCH REPORT

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Application number

EP 84 30 4993

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 4)
A	US-A-4 242 502 (M.R. MALINOW) * Whole document *	1,6	C 07 J 71/00 A 61 K 31/705
D,A	--- CHEMICAL ABSTRACTS, vol. 96, no. 13, 29th March 1982, page 68, no. 97468b, Columbus, Ohio, US; P.K. KINTYA et al.: "Search for hypocholesteremic agents among a group of steroid glycosides", & KHIM.-FARM.ZH. 1981, 15(9), 55-60 * Abstract *	1,6	
D,A	--- JOURNAL OF PHARMACEUTICAL SCIENCES, vol. 67, no. 11, November 1978, pages 1589-1591, American Pharmaceutical Association, Washington, DC, US; R. SEGAL et al.: "Hemolytic properties of synthetic glycosides" * Abstract *	1,6,9	
			TECHNICAL FIELDS SEARCHED (Int. Cl. 4)
A	--- US-A-4 265 886 (K.H. PEGEL) * Claims *	1,6-8	C 07 J 71/00 A 61 K 31/00
D,A	--- FR-A-2 425 859 (ROECAR HOLDINGS) * Claims *	1,6-8	

The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 04-09-1985	Examiner HENRY J.C.
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